CHAPTER ONE

1.1 Background to study

Fruit contains many beneficial qualities to one’s health as they provide an abundance of vitamins, minerals, anti-oxidants and fibres which are all essential for human diet (Klee, 2010). Many people consume fruit juices on a daily basis, as it is becoming an important part of the modern diet in many communities. It acts as a nutritious beverage and can play a significant part in a healthy diet because they offer a variety of nutrients found naturally in fruits (Cleveland, 2006). However, they may also have high sugar content. Although the sugar is natural, it may not be healthy in high quantities. It is suggested that too much sugar could pose harmful health effects as people could develop diabetes, obesity, heart disease, and other complications from excess consumption (Muraki, et al., 2013). Bottled fruit juices is criticized by its concentration or additionally supplemented sugars and contributing the extra calories when consumed in excess (Gormaker, 2008) and in plight of this, companies have contemporarily been marketing fruit juices with “no sugar added” feature in part to address the concern of exceeding the daily recommendation of sugar intake.

The comparison of the sugar content in freshly extracted fruit juice with the sugar content in bottled fruit juice claiming to have “no sugar added” is of interest for a number of reasons. If the sugar content in bottled fruit juice is higher than that of freshly extracted fruit juice, people may want to reconsider the amount of commercially bottled fruit juice they consume.

Fructose is one of the most abundant sugars in fruit juice. Some people believe fructose is healthier than sucrose because it is found naturally in fruit,
however it can be equally harmful (Bazzano, Joshipura, and Hu, 2008). Fructose, also known as fruit sugar, is a simple monosaccharide absorbed directly into the blood stream during digestion. Fruit, vegetables, and honey are all natural sources of fructose. Crystalline fructose, high fructose corn syrup, and sucrose are all three common forms of fructose. Excessive amounts of fructose consumption have been tied to negative health effects. The ever increasing occurrence of obesity, diabetes mellitus, and non-alcoholic fatty liver disease could be a result of excessive fructose intake as well. Also, fructose may promote the formation of glycotoxins, which may contribute to diabetes, the aging process, and the thickening of the arterial walls (Gaby, 2005).

Sugar content varies depending on the type of fruit. All fruit juices contain fructose, but vary in their amount of sucrose, glucose and sorbitol. Fruit with low sugar content include lemon, lime and blackberry. Fruits with low to medium sugar content include papaya, watermelon and apple. Fruits that are considered to have high sugar content include grape, mango, pineapple, and banana. In general, the level of sugar in the juice of a fruit is correlated to the level of sugar in the fruit itself. It is also relevant to know that not all fruits are made into juices. Some of the fruits made into natural and commercially bottled juices include watermelon, apple, orange, grape, pomegranate, pineapple, papaya, cranberry, sugarcane, and lime.

Fruit juice in moderate amounts can help adults meet daily recommendation for fruit consumption, nutrient intake, and calories (Nicklas and Kleinman, 2011). Maintaining the required blood glucose level among adults is of utmost importance, so as to enable all parts of their body (brain, liver, muscle, and heart) function effectively. In light of this, it is important for adults who are
constantly exposed to various stressors which lead to depletion of their energy level, resort to the consumption of fruit juices which is a ready source of energy to maintain their carbohydrate metabolism.

However, adults may prefer fruit juices over whole fruit because they offer good taste and the way fruit juices are packaged friendly in such a way that solve the anaesthetics of adults and as oppose by the strenuous and rigorous processes of peeling, washing, and cutting of whole fruit before their consumption. Juices are the perfect fast food for today’s eat-on-the-run lifestyle.

1.2  Aim

1. To compare the effect of natural and commercial fruit juices on blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

1.3  Objectives

1. To find out if natural and commercial fruit juices has the same effect on blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

2. To find out if there is a significant difference between the effect of natural and commercial fruit juices on blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

1.4  Hypothesis

A.

$H_0$: Natural fruit juice does not increase blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.
\( H_1 \): Natural fruit juice increases blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

B.

\( H_0 \): Commercial fruit juice does not increase blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

\( H_1 \): Commercial fruit juice increases blood glucose level of students in Delta State College of Health Technology, Ofuoma-Ughelli.

1.5 Statement of Problem

Natural fruits are one of the healthiest foods we can consume, relatively loaded with vitamins, minerals and free radical antioxidant. Fruit juices on the other hand are also often perceived as being healthy; this is understandable given that it has the word fruit in it. To date, several studies have been conducted to compare the effects of natural and commercial fruit juices on blood glucose level, but the results have been conflicting and entirely inconsistent. Some studies found that natural and commercial fruit juices does not have the same effect on blood glucose level, while other findings suggested that natural and commercial fruit juices increases blood glucose level and other studies have also found that natural and commercial fruit juices might not equally be associated with increased blood glucose level. Therefore this research work is aimed at comparing the effect of natural and commercial fruit juice on the blood glucose level of students in Delta State College of Health Technology Ofuoma-Ughelli.
CHAPTER TWO

LITERATURE REVIEW

In botany, a fruit is the seed-bearing structure in angiosperms formed from the ovary after flowering. Fruits are the means by which these plants disseminate seeds. Many of them that bear edible fruits, in particular, have propagated with the movements of humans and animals in a symbiotic relationship as a means for seed dispersal and nutrition, respectively; in fact, humans and many animals have become dependent on fruits as a source of food (Lewis, 2002). Fruits account for a substantial fraction of the World’s agricultural output, and some (such as apple, pineapple, and the pomegranate) have acquired extensive cultural and symbiotic meanings.

In common language usage “fruit” normally means the fleshy seeds associated structures of a plant that are sweet or sour and edible in raw state, such as apple, oranges, grapes, bananas, strawberries, lemon, watermelon, and pineapple. In the culinary sense of these words, a fruit is usually any sweet-tasting product, especially those associated with seeds.

2.1 Fruit Juices

Juice is a liquid (drink) that is naturally contained in fruits and vegetables. It can also refer to as liquids that are flavoured with these or other biological food sources such as meat and sea food. It is commonly consumed as a beverage or used as an ingredient or flavouring in foods. It is also a common
practice to mix juices of different fruits and vegetables. Juice did not emerge as a popular beverage of choice until the development of pasteurization methods allowed for the preservation of juice without fermentation (Ryan, 2011). The food and agriculture organization of the United Nations (FAO) estimated the total world production of citric fruit to be about 12,840 318 tonnes in 2012.

2.2 Preparation of Fruit Juice

Juice is prepared by mechanically squeezing or macerating (sometimes referred to as cold pressed) fruit or vegetable flesh without the application of heat or solvents. Example, Orange juice is the liquid that results from pressing the fruit of the plant. Juice may be prepared in the home from fresh fruit and vegetables using a variety of hand or electric juicers. Many commercial juices are filtered to remove fibre or pulp, but high-pulp fresh orange juice is a popular beverage.

Common methods for preservation and processing of fruit juices include canning, pasteurization, concentration, freezing, evaporation and spray drying. Although process methods vary between juices, the general processing method of juices includes;

a. Washing and sorting
b. Juice extraction
c. Straining, filtration and clarification
d. Blending pasteurization
e. Filling dealing and sterilization
f. Cooling, labelling and packing.
After the fruits are picked and washed, the juices are extracted by one of two automated methods. In the first method, two mental cups with sharp mental tubes on the bottom cup come together, removing the peeling and forcing the flesh of the fruit through the mental tube. The peels can then be used further, and as washed to remove oils, which are reclaimed later for usage. The second method requires the fruits to be cut in half before being subjected to reamers which extract the juice.

Then after the juice is filtered, it may be concentrated, which reduces the size of juice by a factor of 5, making it easier to transport and increasing its expiration date. Juices are concentrated by heating under vacuum to remove water and then cooked for 13°C or 30°C. About two thirds of the water in a juice is removed. The juice is then later constituted, in which the concentrate is mixed with water and other factors to return any lost flavour from the concentrating process. Juices can also be sold in a concentrated state, in which the consumer adds water to the concentrated juice as preparation.

Juices are then pasteurized and filtered into a container, often while still hot. If the juice is poured into a container while hot, it is cooled as quickly as possible. Packages that cannot stand heat require sterile conditions for filling. Chemicals such as hydrogen peroxide can be used to sterilize containers.

2.3 Glucose

Glucose is the most important sugar in human metabolism and is one of a group of carbohydrate known as simple sugar. Glucose contains six atom of carbon, twelve atom of hydrogen, and six atom of oxygen giving it a chemical formula of C₆H₁₂O₆. The name “glucose” comes from the Greek word glycols
meaning “sweet wine”. The suffix “-ose” is a chemical classifier, denoting a carbohydrate (Thenare, et al., 1838). Alternate names of glucose include grape, sugar, and corn sugar.

Glucose provides the energy for life processes. It is the main end product of carbohydrate digestion. Oxidation by the glycolytic and triglycolytic pathway provides the chemical energy needed for cellular activity. All parts of our body (muscle, heart, brain, liver, etc) need energy to work irrespective of what we are doing even when we are sleeping; our bodies depend on glucose to function effectively.

When glucose is not required for the body’s immediate energy needs, it is converted to glycogen and stored in the liver and muscles (glycogenesis). Muscle glycogen provides the glucose of muscular activity. Excess glucose is oxidized to fatty acid and stored as fat in tissues. If needed, glucose can also be formed from fats and protein (gluconeogenesis). An increase in the breakdown of fats to provide energy results in an increase in the production of ketone (Monica, 2005).

Glucose is the most important medical analyte measured in blood sample. It is found in the human blood stream where it is referred to as “blood glucose”. The normal concentration of glucose in the blood is about 0.1%, but it becomes much higher in persons suffering from diabetes mellitus (Noda, et al., 2013).

2.4 Role and Functions of Glucose

The roles of glucose in the body includes providing energy for working muscles, providing fuel for the central nervous system, enabling fat metabolism
and preventing protein from being used as a source of energy. Glucose is the preferred source of energy for muscle contraction and biological work. Foods containing glucose are in grains, fruits and milk groups. Vegetables have a small amount of glucose. After foods containing glucose is eaten, they are broken down to simple units of sugar in the stomach and small intestine. These units of sugar are absorbed in the small intestine and then enter the blood stream where they travel to the liver. Fructose and galactose are converted to glucose by the liver.

Glucose spares the use of protein as an energy source. When glucose consumption is inadequate protein is broken down to glucose in order to maintain a constant blood glucose level. However, when proteins are broken down they lose their primary role as building blocks for muscles. In addition, protein breakdown may result in an increased stress on the kidney, where protein by product is excreted into the urine.

Finally, glucose is essential for the central nervous system. The brain primarily uses glucose as its energy source, and a lack of glucose can result in weakness, dizziness, and a low blood glucose level (hypoglycaemia). Reduced blood glucose level during exercise decreases performance and could lead to mental as well physical fatigue.

2.5 Glycolysis

Use of glucose as an energy source in cells is aerobic respiration, anaerobic respiration or fermentation. All of these processes follow from an earlier metabolic pathway known as glycolysis. The first step of glycolysis is the phosphorylation of glucose by a hexose to form glucose-6-phosphate. The main reason for the immediate phosphorylation of glucose is to prevent its diffusion
from the cell as the charged phosphate group prevents the glucose-6-phosphate from easily crossing the cell membrane. Furthermore, addition of high energy phosphate group activates glucose for subsequent breakdown in later steps of glycolysis. At physiological conditions the initial reaction is irreversible.

In anaerobic respiration, one glucose molecule is a net gain of two ATP molecules (four ATP molecules are produced during glycolysis, but two are required by enzymes used during the process) (Salway, 2006). In aerobic respiration, a molecule of glucose is much more profitable in that a maximum net product of 30 or 32 ATP molecules (depending on organism) is generated.

2.6 Glucoregulation

Glucoregulation is the maintenance of steady levels of glucose in the body; it is part of homeostasis and also keeps a constant internal environment around cells in the body. The hormone insulin is the primary regulatory signal in animals suggesting that the basic mechanism is very old and very central to animal life. When present, it causes many tissues cells to take up glucose from the circulation and cause some cells to take in and hold lipids, and in many cases controls cellular electrolyte balance and amino acid uptake as well. Its absence turns off glucose uptake into cells, reverses electrolyte adjustment, begins glycogen breakdown and glucose release into the circulation by some cells, begins lipid release from lipid storage cells etc.

The level of circulatory glucose (known informally as “blood sugar”) is the most important signal to the insulin producing cells, because the level of circulatory glucose is largely determined by the intake of dietary carbohydrate, diet control major aspect of glucose metabolism via insulin. In humans, insulin is
made by beta cells in the pancreas, fat is stored in adipose tissue cells and glycogen is both stored and released as needed by liver cells. Regardless of insulin levels, no glucose is released to the blood from internal glycogen stores from muscle cells.

The hormone glucagon, on the other has an effect opposite to that of insulin, forcing the conversion of glycogen in cells to glucose, which is then released into the blood stream. Muscle cells however lack the ability to export glucose into the blood. Other hormones notably growth hormone, corticol, and catecholamine have glycol-regulatory actions similar to glycogen.

### 2.7 Production of Glucose

Glucose is produced commercially via the enzymatic hydrolysis of starch. Many crops can be used as the source of starch. Maize, rice, wheat, cassava, corn husk and sago are all used in various parts of the world. Most commercially glucose occurs as a component of invert sugar, a roughly 1:1 mixture of glucose and fructose. In principle, cellulose could be hydrolysed to glucose, but this process is not yet commercially practical (Nassif, Hassan, and Sawsan, 2009). Glucose has approximately 75% the sweetness of sucrose (McMurry, 2009).

### 2.8 Food Sources of Glucose

Glucose is mostly found in food as a building block in more complex carbohydrates. Complex carbohydrates are composed of thousands of glucose units linked together in chains. Our digestive system breaks down complex carbohydrates into many molecules for use by our body cells to create energy. Some of the food source of glucose includes;
a. **Fruit Juice**: Fruit juice like grape, mango, pineapple, watermelon, mango, pomegranate, etc are high in glucose (around 20g/100g). The glucose content in canned bottled fruit juice tends to be higher than freshly squeezed unstrained juices.

b. **Vegetables**: Vegetables contain glucose often in the form of starch. Starch is the energy storage molecule of plants. It is formed by long chains of a glucose molecule linked together.

c. **Fruit**: Virtually all kinds of fruits contain glucose. For example, banana an on-the-go fruit is a good source of fibre, potassium and vitamin C, and has a medium range on the glycemic index. Grape, mango, pineapples, pomegranate etc are equally good sources of glucose.

### 2.9 Analysis

Blood glucose is usually measured on a protein-free filtrate of whole blood. This normally gives a value lower than that of plasma due to the presence of red blood cell (RBC). However, most methods make use of plasma instead of serum or whole blood. Glucose estimation using plasma or whole blood requires the use of an anticoagulant. The fluoride oxalate mixture used for this purpose is composed of 2mg of sodium fluoride and 6mg of potassium oxalate per ml of blood. More than 5mg of sodium fluoride per ml of blood acts as an inhibitor for the enzymatic estimations.

Collection of specimen for measurements of blood glucose level should be done on the day and time requested. This is because collection time is usually related to food intake, insulin treatment, or both. It may be necessary sometimes
to note the time of blood collection on the specimen container and on the patient’s request form.

Fasting specimen refers to blood collected after a period of no food intake. For adults, fasting time usually 10 to 16 hours; while for children, it is 6 hours. Post-prandial specimen refers to blood collected usually 2 hours after a meal has been eaten. Random specimen refers to blood sample collected at any time regardless of food consumption.

**Methods:**

*a. Oxidase Peroxidase Method (D. Muller, 1928)*

**Principle:** Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide, in the presence of the enzyme peroxidase is broken down and the oxygen given off reacts with 4-aminophenazone and phenol to give a pink colour measured at a wavelength of 540 nm.

**Advantages**

The method allows for precision and accuracy.

The method is specific for glucose so no interference

Method is ideal for a busy laboratory

A wide range of anticoagulant in combination with sodium fluoride can be used.
Disadvantages

Error could occur from standard if enough time is not allowed for mutarotation to take place.

b. Copper Reduction (Shaffer, P.A. and Hartmann, A.F. 1920)

Principle: Glucose in hot alkaline solution will reduce Copper two ions which will reduce phosphomolybdic acid to molybdenum, a blue coloured substance that can be measured spectrophotometrically at 680nm. The intensity of the colour produced is proportional to sugar present.

Advantage:

The method is simple and cheap.

Disadvantages:

The reaction is not stoichiometric since it depends on alkalinity, time and temperature of heating and concentration of reagent.

The method is not specific for glucose

The method is not suitable for urine glucose estimation due to the large quantity of creatine and uric acid.

c. Reduction of Cupric to Cuprous Salt (Folin, O. and Wu H. 1919)

This is one of the oldest methods for estimation of blood sugar. However, it is still very much in use in countries where enzyme preparations are not easy to obtain.

Principle: The method is based on three stages;
i. Precipitation of blood proteins with copper tungstate.

ii. Reduction of cupric sulphate to cuprous oxide.

iii. Colorimetric measurement of the subsequent blue-green colour produced on the addition of molybdate reagent to the cuprous oxide.


This is a simple enzyme method in which oxidase peroxidise enzyme and the colour reagent are impregnated on the reagent strips. This method uses a reflometric meter to register the amount of glucose in the blood.

**Technique:** A drop of capillary blood is placed in the test strip which after the prescribed time interval is wiped and inserted into the instrument. The colour change is read in a reflectance meter on which the result in mg/dl is visualised.

**Advantages**

They are simple and reliable.

They have excellent correlation with reference method.

They give good precision and accuracy.

The meter is automatically calibrated by the bar code on each test strip;

They can measure values as high as 450mg/dl

e. O-Toludine (Hultman, 1959)

This method makes use of the principle that glucose reacts specifically with o-toluidine an aromatic amine in glacial acetic acid at 100 degree Celsius to produce a blue–green colour. This colour can be measured colorimetrically. The method has the additional advantage that it can be performed directly on serum,
plasma or urine with deproteinization. The o-toluidine method is no longer widely used today because o-toluidine is believed to be a carcinogen.

f. Alkaline Ferricyanide Method (Bulger H.P, and Johns H.E, 1941)

**Principle:** Glucose will reduce yellow ferricyanide ion to colourless ferrocyanide in hot alkaline solution. The decrease in the intensity of yellow measured at 420nm is proportional to the blood glucose concentration.

**Advantages**

The unreduced ferricyanide can be measured iodometrically with kl titration.

The reagents are stable and cheap.

The method can be automated

**Disadvantages**

The method is not specific for glucose.

The use of high blank absorbance introduces error.

Ferricyanide is a highly poisonous substance.

**Reference Range**

Fasting blood glucose level:

For adults = 3.3-5.5mmol/L or 60-100mg/dl

For children = 2.5-5.0mmol/L or 45-90mg/dl

Post-prandial blood glucose = Up to 6.9mmol/L or up to 125mg/dl
Random blood glucose =3.3=7.4mmol/L or 60-133mg/dl

2.10 Comparison of Fruit Juice to Whole Fruit

Longitudinal prospective cohort studies conducted at Havard showed a significantly increased on blood glucose level when juices were consumed compared to whole fruits (Muraki, et al., 2013). Fruit has also been linked to childhood obesity (Wojcicki, et al., 2012). Since juices do not contain fiber from the plants, many further benefits are therefore negated found in whole fruit instead. Prebiotics have marked beneficial impact on calcium absorption, mineral absorption, bowel health, and promoting a healthier gut flora (Kumar, et al., 2012). Further more, most polyphenols are bound to the plant fibers and constitute the major portion of dietary phytonutrients (Arranz, 2010). There is therefore marked reduction in nutritional benefit from consuming fruit juice compared to the consumption of whole fruits.

2.11 Health Effects of Natural Fruit Juices and Uses

Many fruits are used to make beverages, such as fruit juices (orange juice, pineapple juice, grape juice, etc) or alcoholic beverages, such as wine, fruit beer and brandy. Natural fruit juices are packed with live enzymes and vitamins. Carrot juices for example are full of the nutrient; beta-carotene (which converts into vitamin A in the body), and green juices give us amazing doses of chlorophyll. Freshly made juices are alkalising and they provide our bodies with intense hydration and oxygenated cells and bloodstream.

Freshly extracted fruit juices are generally high in water, vitamin C, minerals and free radical antioxidant (Liu, 2014). Regular consumption of natural
fruit juices is generally associated with reduced risk of several disease and functional declines associated with aging (Wang, et al., 2014).

2.12 Health Effects of Commercial Fruit Juices

Commercial fruit juices are often consumed for their perceived health benefits. For example, orange juice is rich in vitamin C, folic acid, and potassium; it is an excellent source of bio-available phytochemicals (Franke, Cooney, and Cluster, 2005) and significantly improves blood lipid profiles in people affected with hypercholesterolemia (Daher, Khalil, and Barody, 2005). Prune juice is associated with a digestive health benefit. Cranberry juice has long been known to help prevent or even treat bladder infections, and it is now known that a substance in cranberry prevents bacteria from binding to the bladder.

Many bottled fruit juices have high sugar content than sweetened soft drinks; e.g. typical grape juices have 50% more sugar than coca cola (Bazzanno, et al., 2008). While soft drinks cause oxidative stress when ingested and may even lead to insulin resistance in the long term, the same thing cannot be attributed to fruit juices. On contrary, fruit juices are actually known for their ability to raise serum antioxidant capacity and even offset the oxidative stress and inflammation normally caused by high fat and high sugar meals (Ghanim, et al., 2010). However, frequent consumption of fruit juices causes’ dental decay, and may be a more significant factor in the development of dental caries (cavities) than eating candy (NHS, 2014). Fruit juice causes dental decay because it naturally contains acids which chemically dissolve the enamel off the surface of the tooth, and sugars that the bacteria in the mouth, frequent to create even more tooth destroying acids.
During the course of production of commercially bottled fruit juice, dietary fibres present in the fruit are filtered out. In other cases, other ingredients are added (Norris, and Kleinman, 2009). High fructose corn syrup an ingredient in many commercial bottled fruit juice cocktails has been linked to the increased incidence of type 2 diabetes. High consumption of juice is also linked to weight gain in some studies (Andrea, et al., 2006) but not in others (O’Neil, Nicklas, and Zanovec, 2010). Fruit juice in moderate amounts can help children and adults meet daily recommendations for fruit consumption, nutrient intake and calories (Zanovec, Nicklas, and Fulgani, 2012).

2.13 Effects of Natural and Commercial Fruit Juice on Blood Glucose Level

According to the research carried out in the United States by Muraki et al. (2012), it was concluded that natural fruit juices generally lowers blood glucose level in blood, while commercial fruit juices increases blood glucose level. The association with the risk of increased blood glucose in individuals differed significantly among individual fruits (P> 0.001). While all natural fruit juices were shown to reduce the level of glucose, the fruit (grapes, pineapple, and blueberries) appeared to be particular effective. It was however found in this study that consumption of commercially bottled fruit juice is associated with increase blood glucose level. The difference between both fruit juices type were not accounted for by variation in the glycemic/glycemic load value of the fruit juices.

According to a research study that was carried out in the United States by Jasmine, (2012), on the determination of the sugar content in bottled fruit juice
with a “no sugar added” label and that of fresh fruit juices, the data gotten demonstrated experimentally no statistical difference (P< 0.05). The result suggested that sugar content in the commercially bottled fruit juice with a “no sugar added” label is an accurate representation of sugar content in freshly extracted juice of the corresponding fruit. It was also found in this study that both natural and commercial fruit juices correspondingly increase blood glucose level.

According to a research study that was carried out in the United States by Bazzano et al. (2008), discovered that among participants (females between the age of 30-55 years) consuming >3 cups of apple juice per month, the health risk was 1.15 (P> 0.001). The corresponding health risk for grape juice consumers was 1.14 (P< 0.001). The median intake of fruit in this population was 1.08 servings per day. According to the findings in this research work, it was concluded that fruit juice intake was associated with an increased blood glucose level (1.18[1.10 = 1.25]), while natural fruit juices was associated with a lower increase in blood glucose level.

Another research work carried out in China by Ravanshad et al. (2014), it was found that subjects who consumed 240ml of sour orange juice daily for 4 months, experienced a significant decrease in their fasting blood glucose level (P< 0.05). It was therefore concluded that short term incorporation of sour orange juice in diabetics’ diet had a lowering effect on fasting blood glucose level (P <0.05). Subgroup analyses further suggested that the effect of fruit juice on blood glucose concentration was not influenced by population region baseline glucose concentration, duration, type of fruit juice, glycemic index of fruit juices, fruit juice nutrient constitution, and total polyphenols.
According to a research study carried out in Japan by Enab, *et al.* (2013), comparing the effect of fruit juices on blood glucose level of the participants (Japanese men and women aged 30 years and above) found that increased blood glucose level was not associated with either gender (\(P >0.05\)). Fruit juices were found to be associated with more increase glucose concentration in women but not men. The association was evident of overweight, highly educated and premenopausal women, and women with collar job.

Lastly, according the research study carried out in London by Cooper *et al.* (2012) studied the association between natural and commercial fruit juices and found both juices increase blood glucose level. These findings suggest that diet characterized by a greater variety of both natural and commercial fruit juices is associated with increased blood glucose level.

### 2.14 Conclusion

While freshly extracted fruit juices and commercially produced fruit juices can be a good source of vitamins, minerals, antioxidants, including very potent flavonoids, one key property of these fruit juices are the fact that they tend to be very sugary indeed. Many fruit juices have a sugar concentration similar to sugar sweetened beverages. And some juices (e.g. grape juice) contain considerable more sugar than this even.

Drinking fruit juice is associated with diabetes risk, but they cannot prove that fruit juices can cause diabetes. However, the high sugar nature of fruit juices means that they may indeed have a genuine diabetes inducing effects. It is sometimes said that the fruit sugar (fructose) found in high levels in fruit juice is relatively harmless, an account of the fact that it does not tend to raise blood glucose level. But, fructose in anything is safe, and its consumption has been
found to induce something known as “insulin resistance”, which is a precursor of diabetes mellitus.
CHAPTER THREE

MATERIALS AND METHOD

3.1 Research Design

Experimental design was employed.

3.2 Population Area

The population area of this study was Delta State College of Health Technology, located in Ofuoma Community of Ughelli North Local Government area in Delta Central senatorial district of Delta, Delta State. The College is bounded in the North by Kokori Community in the south by Afiesere community, in the West by Isokolo community and in the East by Agbara Community.

3.3 Population of Study

The population of study was Department of Medical Laboratory Technology and Community Health students of Delta State College of Health Technology, Ofuoma-Ughelli, who were between the age range of 18years and above. The total population that was considered for this study was 100 students from the two departments in the College.

3.4 Sample Size and Sampling Technique

Random sampling technique was used to select students from both Medical Laboratory and Community Health Department of the College. The average numbers of students in Community Health Department are 250; the
researcher selected one in every five students (1:5) students and obtained 50 subjects. For Medical Laboratory Technology Department having an average number of students of 150, the researcher selected one in every three students (1:3) and also obtained 50 subjects. The 100 students obtained were further grouped into two groups A and B and were administered with natural and commercial fruit juices respectively.

3.5 Sample Collection

On the first day, 2mls of blood sample was collected into a well labelled fluoride oxalate container (fasting sample), and 250ml of natural and commercial fruit juice was administered to subjects in group A and B respectively for one week of the study. 2mls of blood sample was collected into a well labelled fluoride oxalate container 2 hours after the consumption of natural and commercial fruit juice on the last day. The sample was taken to the laboratory for analysis.

3.6 Laboratory Analysis

Enzymatic method was employed for this research.

a. Principle (Glucose Oxidase Method)

Glucose oxidase catalyzes the enzymatic oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator which is measured spectrophotometrically at a wavelength of 500nm.
3.6.1  Procedure for Analysis

1. Set up three test tubes as follows; blank, standard and test.
2. Preparation of blank: Pipette 1ml of working solution into the first test tube and 0.01ml of distilled water into the tube.
3. Preparation of standard: Pipette 1ml of working solution into the second test tube and 0.01ml of the standard solution into the test tube.
4. Preparation of test: Pipette 1ml of working solution into the third test tube and 0.01ml of patient plasma into the test tube.
5. Mix well and incubate all test tubes at 37°C for 10 minute in an incubator. Shake occasionally.
6. Remove from the incubator; allow cooling and measuring the absorbance within 30 minutes.
7. Set the zero with the blank.
8. The absorbance of the test solution is read colorimetrically at 500nm.

3.6.2  Manual Calculation

\[
\text{Glucose concentration (mmol/l)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \frac{\text{Concentration of standard}}{1}
\]

3.6.3  Result

Fasting blood glucose level:

\[3.3 – 5.6 \text{mmol/l or } 60 – 100 \text{mg/dl}\]

Random blood glucose:

\[3.3 – 7.4 \text{mmol/l or } 60 – 133 \text{mg/dl}\]
**Ethical Permission**

Ethical permission was obtained from the Ethical Committee of the College.

**Statistical Analysis**

Data were analysed using Student T-test.
CHAPTER FOUR

4.1 RESULTS

Table 4.1 shows the result of the grand mean and standard deviation ratings of both the control and the tested group. The mean ± standard deviation of the control group is 76.06 ± 20.89, while that of the test group is 82.26 ± 18.77 respectively. The table also shows Student T-test statistics used to analyze the effect of natural fruit juice on blood glucose level. The table revealed a calculated T-value of 1.56, less than the T-critical value of 1.960 (P > 0.05). We therefore fail to reject the null hypothesis and conclude that natural fruit juice does not increase blood glucose level.

Table 4.2 shows the result of the grand mean and standard deviation ratings of both the control and the tested group. The mean ± standard deviation of the control group is 60.58 ± 31.36, while that of the test group is 87.2 ± 58.12 respectively. The table also shows Student T-test statistics used to analyze the effect of commercial fruit juice on blood glucose level. The table revealed a calculated T-value of 2.85, greater than the T-critical value of 1.960 (P <0.05). We therefore reject the null hypothesis and conclude that commercial fruit juice increases blood glucose level.
### 4.1 T-test Table of Natural Fruit Juice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number Sample Tested</th>
<th>Mean± SD</th>
<th>Calculated Value</th>
<th>Critical Value</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>76.06 ± 20.89</td>
<td>1.56</td>
<td>1.960</td>
<td>P &gt;0.05 Insignificant</td>
</tr>
<tr>
<td>Test</td>
<td>50</td>
<td>82.26 ± 18.77</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 4.2 T-test Table for Commercial Fruit Juice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number Sample Tested</th>
<th>Mean± SD</th>
<th>Calculated Value</th>
<th>Critical Value</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>60.58 ± 31.36</td>
<td>2.85</td>
<td>1.960</td>
<td>P &lt;0.05 Significant</td>
</tr>
<tr>
<td>Test</td>
<td>50</td>
<td>87.2 ± 58.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FIVE

DISCUSSION

The result of our study shows that natural fruit juice does not increase blood glucose level. This is evident in the T-value of 1.56, less than the T-critical value of 1.960 (P > 0.05). Our result corresponds with that of other studies carried out to compare the effect of natural fruit juice on blood glucose level. In one of such study by Muraki, et al. (2013), it was suggested that natural fruit juice decreases blood glucose level (P >0.05). The study of Bazzano, et al. (2008), found that consuming natural fruit juice of the corresponding fruit was associated with reduced effect on blood glucose level (P >0.05). The reason for the decrease in blood glucose level maybe accounted for by the freshness and absence of additionally supplemented sugars and preservatives.

In contrast, the study by Wang, et al. (2012) reported that consumption of natural fruit juices did not show a significant decrease in the impact of natural fruit juices on blood glucose level of participants (P <0.05). Another study by Enab, et al. (2013), suggested that natural fruit juices increases blood glucose level (P <0.05). Also, the study by Cooper, et al. (2012) found the association between natural fruit juices and blood glucose level, did not show a significant reduction in blood glucose level of participants (P <0.05).

Our study also found that commercial fruit juices increases blood glucose level (P <0.05). Our findings is in agreement with the study by Enab, et al. (2013), who suggested that commercial fruit juice significantly increase blood glucose level (P <0.05). The findings of Jasmine, (2012) found that consumption of commercial fruit juice increase blood glucose (P <0.05). Our findings is not in
line with the result of the study carried out by Muraki, et al. (2013) and Bazzano, et al. (2008), who both reported that consumption of commercial fruit juice was found to significantly increase blood glucose level (P >0.05). The reason for the increase in blood glucose level by commercial fruit juices maybe because of the additionally supplemented sugars and preservatives added by manufacturers of commercially bottled fruit juices.

The reason for the variation of our result with other studies may be because of some predisposing factors such as age, lifestyle, location, diet, occupation, social and economic status, glycemic load of fruit juices, menopausal status, oral contraceptive use, etc.

Conclusively, juicing extracts the juice from fresh fruits. The resulting liquid contains most of the vitamins, minerals and plant chemicals (phytonutrient) found in whole fruit. However, whole fruits also have healthy fiber which is lost during juicing and accounts for rapid absorption and increase in blood glucose level when natural fruit juices are consumed. If a commercially produced juice is to be bought from a juicing stand or store, pasteurized product should be selected. It should be also kept in mind that these juices may contain more sugar than we think since they do not contain fibres and may have been supplemented with sugars and preservatives which tend to increase blood glucose level significantly.
REFERENCES


Variety of Fruit and Vegetable Intake and Incident of Type 2 Diabetes. 


APPENDIX I

Reaction Principle

Glucose + Oxygen + Water $\xrightarrow{\text{Glucose Oxidase}}$ Gluconic acid + Hydrogen peroxide

Hydrogen peroxide + 4-aminophenazone + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + Water
APPENDIX II

Preparation of Reagent

**R1a.** Glucose reagent (dried) powder (13.8mg/dl).

**R1b.** Buffer solution (100ml).

Mix the R1a and R1b to form working solution. Store at 2-8°C.
## APPENDIX III

### Summary of Procedure:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Working solution</strong></td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td>0.01ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Standard solution</strong></td>
<td>-</td>
<td>0.01ml</td>
<td>-</td>
</tr>
<tr>
<td><strong>Test sample (plasma)</strong></td>
<td>-</td>
<td>-</td>
<td>0.01ml</td>
</tr>
</tbody>
</table>
APPENDIX IV

Material Required

Materials that will be used for this study include;

i. Tourniquet
ii. 70% alcohol
iii. Cotton wool
iv. Syringe and needle
v. Fluoride oxalate container
vi. Centrifuge
vii. Pasteur pipette and micro pipette
viii. Randox glucose reagent
ix. Incubator
x. Colorimeter
xi. Hand gloves
APPENDIX V

\( \bar{x} = \text{Mean} \)

\( N = \text{Sample size number of participants} \)

\( \Sigma = \text{Sum} \)

\( DF = \text{Degree of Freedom} \)

\( SD = \text{Standard Deviation} \)

\[
t = \frac{X_1 - X_2}{\sqrt{\left[\frac{\Sigma x_1^2 + \Sigma x_2^2}{n_1 + n_2}\right]\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}}
\]
APPENDIX VI

Summary of results showing the Effect of Natural Fruit juice on Blood Glucose Level

<table>
<thead>
<tr>
<th>CONTROL GROUP</th>
<th></th>
<th>TEST GROUP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X</strong></td>
<td><strong>X₁[× – x̄]</strong></td>
<td><strong>X₁[× – x̄]²</strong></td>
<td><strong>X</strong></td>
</tr>
<tr>
<td>80</td>
<td>3.94</td>
<td>15.52</td>
<td>87</td>
</tr>
<tr>
<td>73</td>
<td>-3.06</td>
<td>9.36</td>
<td>62</td>
</tr>
<tr>
<td>80</td>
<td>3.94</td>
<td>15.52</td>
<td>83</td>
</tr>
<tr>
<td>127</td>
<td>50.94</td>
<td>2594.88</td>
<td>130</td>
</tr>
<tr>
<td>80</td>
<td>3.94</td>
<td>15.52</td>
<td>77</td>
</tr>
<tr>
<td>90</td>
<td>13.94</td>
<td>194.32</td>
<td>103</td>
</tr>
<tr>
<td>73</td>
<td>-3.06</td>
<td>9.36</td>
<td>63</td>
</tr>
<tr>
<td>80</td>
<td>3.94</td>
<td>15.52</td>
<td>96</td>
</tr>
<tr>
<td>53</td>
<td>-23.06</td>
<td>531.76</td>
<td>53</td>
</tr>
<tr>
<td>50</td>
<td>-26.06</td>
<td>679.12</td>
<td>77</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>74</td>
</tr>
<tr>
<td>57</td>
<td>-19.06</td>
<td>363.28</td>
<td>100</td>
</tr>
<tr>
<td>63</td>
<td>-13.06</td>
<td>170.56</td>
<td>70</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>100</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>83</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>65</td>
</tr>
<tr>
<td>103</td>
<td>26.94</td>
<td>725.76</td>
<td>100</td>
</tr>
<tr>
<td>113</td>
<td>36.94</td>
<td>1364.56</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>83</td>
<td>6.94</td>
<td>48.16</td>
<td>86</td>
</tr>
<tr>
<td>83</td>
<td>6.94</td>
<td>48.16</td>
<td>80</td>
</tr>
<tr>
<td>70</td>
<td>-6.06</td>
<td>36.72</td>
<td>80</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>76</td>
</tr>
<tr>
<td>77</td>
<td>0.94</td>
<td>0.88</td>
<td>93</td>
</tr>
<tr>
<td>73</td>
<td>-3.06</td>
<td>9.36</td>
<td>80</td>
</tr>
<tr>
<td>120</td>
<td>43.94</td>
<td>1930.72</td>
<td>77</td>
</tr>
<tr>
<td>70</td>
<td>-6.06</td>
<td>36.72</td>
<td>86</td>
</tr>
<tr>
<td>90</td>
<td>13.94</td>
<td>194.32</td>
<td>80</td>
</tr>
<tr>
<td>53</td>
<td>-23.06</td>
<td>531.76</td>
<td>70</td>
</tr>
<tr>
<td>93</td>
<td>16.94</td>
<td>286.96</td>
<td>77</td>
</tr>
<tr>
<td>116</td>
<td>39.94</td>
<td>1595.20</td>
<td>120</td>
</tr>
<tr>
<td>126</td>
<td>49.94</td>
<td>2494.00</td>
<td>130</td>
</tr>
<tr>
<td>87</td>
<td>10.94</td>
<td>119.68</td>
<td>60</td>
</tr>
<tr>
<td>50</td>
<td>-26.06</td>
<td>679.12</td>
<td>60</td>
</tr>
<tr>
<td>77</td>
<td>0.94</td>
<td>0.88</td>
<td>67</td>
</tr>
<tr>
<td>97</td>
<td>20.94</td>
<td>438.48</td>
<td>70</td>
</tr>
<tr>
<td>97</td>
<td>20.94</td>
<td>438.48</td>
<td>114</td>
</tr>
<tr>
<td>87</td>
<td>10.94</td>
<td>119.68</td>
<td>77</td>
</tr>
<tr>
<td>93</td>
<td>16.94</td>
<td>286.96</td>
<td>97</td>
</tr>
<tr>
<td>77</td>
<td>0.94</td>
<td>0.88</td>
<td>78</td>
</tr>
<tr>
<td>56</td>
<td>-20.06</td>
<td>402.40</td>
<td>60</td>
</tr>
<tr>
<td>50</td>
<td>-26.06</td>
<td>679.12</td>
<td>60</td>
</tr>
<tr>
<td>77</td>
<td>0.94</td>
<td>0.88</td>
<td>73</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>70</td>
<td>-6.06</td>
<td>36.72</td>
<td>86</td>
</tr>
<tr>
<td>60</td>
<td>-16.06</td>
<td>257.92</td>
<td>80</td>
</tr>
<tr>
<td>53</td>
<td>-23.06</td>
<td>531.76</td>
<td>74</td>
</tr>
<tr>
<td>60</td>
<td>-16.06</td>
<td>257.92</td>
<td>93</td>
</tr>
<tr>
<td>67</td>
<td>-9.06</td>
<td>82.08</td>
<td>73</td>
</tr>
<tr>
<td>53</td>
<td>-23.06</td>
<td>531.76</td>
<td>66</td>
</tr>
<tr>
<td>53</td>
<td>-23.06</td>
<td>531.76</td>
<td>60</td>
</tr>
<tr>
<td>83</td>
<td>6.94</td>
<td>48.16</td>
<td>130</td>
</tr>
<tr>
<td>(\frac{\sum x_1}{n_1} = \frac{3803}{50}) &amp; (\sum x_1^2 = 21374.82) &amp; (\frac{\sum x_2}{n_2} = \frac{4113}{50}) &amp; (\sum x_2^2 = 17263.62)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[t = \frac{X_1 - X_2}{\sqrt{\left(\frac{\sum x_1^2 + \sum x_2^2}{n_1 + n_2 - 2}\right)\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}}\]

\[t = \frac{76.06 - 82.26}{\sqrt{\left[\frac{21374.82 + 17263.62}{50 + 50 - 2}\right]\left[\frac{1}{50} + \frac{1}{50}\right]}}\]

\[t = \frac{6.2}{\sqrt{\left[\frac{38638.44}{98}\right]\left[\frac{1}{50} + \frac{1}{50}\right]}}\]
\[
t = \frac{6.2}{\sqrt{[394.27][0.04]}}
\]

\[
t = \frac{6.2}{\sqrt{[15.77]}}
\]

\[
t = \frac{6.2}{3.97} = 1.56
\]

Df. = 98

Table value = 1.960
### APPENDIX VII

Summary of results showing the Effect of Commercial Fruit juice on Blood Glucose level

<table>
<thead>
<tr>
<th>CONTROL GROUP</th>
<th>TEST GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X$</td>
<td>$X_1[\times - \bar{x}]$</td>
</tr>
<tr>
<td>63</td>
<td>2.42</td>
</tr>
<tr>
<td>60</td>
<td>-0.58</td>
</tr>
<tr>
<td>63</td>
<td>2.42</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>47</td>
<td>-13.58</td>
</tr>
<tr>
<td>80</td>
<td>19.42</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>57</td>
<td>-3.58</td>
</tr>
<tr>
<td>43</td>
<td>-17.58</td>
</tr>
<tr>
<td>60</td>
<td>-0.58</td>
</tr>
<tr>
<td>120</td>
<td>59.42</td>
</tr>
<tr>
<td>80</td>
<td>19.42</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>110</td>
<td>49.42</td>
</tr>
<tr>
<td>93</td>
<td>32.42</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
</tr>
<tr>
<td>90</td>
<td>29.42</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>47</td>
<td>-13.58</td>
</tr>
<tr>
<td>44</td>
<td>-16.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>43</td>
<td>-17.58</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>54</td>
<td>-6.58</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>46</td>
<td>-14.58</td>
</tr>
<tr>
<td>45</td>
<td>-15.58</td>
</tr>
<tr>
<td>44</td>
<td>-16.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>50</td>
<td>-10.58</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>43</td>
<td>-17.58</td>
</tr>
<tr>
<td>60</td>
<td>-0.58</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>237</td>
<td>176.42</td>
</tr>
<tr>
<td>46</td>
<td>-14.58</td>
</tr>
<tr>
<td>40</td>
<td>-20.58</td>
</tr>
<tr>
<td>53</td>
<td>-7.58</td>
</tr>
<tr>
<td>80</td>
<td>19.42</td>
</tr>
<tr>
<td>57</td>
<td>-3.58</td>
</tr>
<tr>
<td>85</td>
<td>24.42</td>
</tr>
<tr>
<td>84</td>
<td>23.42</td>
</tr>
</tbody>
</table>

$$\sum x_1 = \frac{3029}{50}$$

$$\bar{x} = 60.58$$

$$\sum x_1^2 = 48202.18$$

$$\sum x_2 = \frac{4360}{50}$$

$$\bar{x} = 87.2$$

$$\sum x_2^2 = 165492$$

$$t = \frac{X_1 - X_2}{\sqrt{\frac{\sum x_1^2 + \sum x_2^2}{n_1 + n_2 - 2} \left[ \frac{1}{n_1} + \frac{1}{n_2} \right]}}$$

$$t = \frac{60.58 - 87.2}{\sqrt{\frac{48202.18 + 165492}{50 + 50 - 2} \left[ \frac{1}{50} + \frac{1}{50} \right]}}$$

$$t = \frac{26.62}{\sqrt{\frac{213694.2}{98} \left[ \frac{1}{50} + \frac{1}{50} \right]}}$$
\[ t = \frac{26.62}{\sqrt{[394.27][0.04]}} \]

\[ t = \frac{26.62}{\sqrt{[87.22]}} \]

\[ t = \frac{26.62}{9.34} = 2.85 \]

\[ \text{Df.} = 98 \]

**Table value** = 1.960